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(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF EPSTEIN-BARR VIRUS-ASSOCIATED DISEASES

(57) Abstract

Antisense oligonucleotides which inhibit the replication of Epstein-Barr virus are provided. Compositions and methods for the treatment of Epstein-Barr virus-associated diseases are also provided.

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COMPOSITIONS AND METHODS FOR TREATMENT OF EPSTEIN-BARR VIRUS-ASSOCIATED DISEASES

This application is a continuation-in-part of U.S. Patent Application Serial No. 485,297, filed February 26, 1990 (ISIS-0009).

FIELD OF THE INVENTION

This invention relates to the design and synthesis of antisense oligonucleotides which can be administered to inhibit the replication of Epstein-Barr virus and treat Epstein-Barr virus-associated disease. These compounds can be used either prophylactically or therapeutically to reduce the severity of diseases caused by Epstein-Barr virus. Oligonucleotides which are specifically hybridizable with RNA targets are described.

BACKGROUND OF THE INVENTION

Epstein-Barr virus (EBV) was first discovered in the early 1960's in the course of a search for the causative agent for a lymphoma, now called Burkitt's lymphoma, which occurred with high incidence among children in certain parts of Africa. Cell lines established from Burkitt's lymphoma tumors were found to contain the virus, which was also found in lymphoid cell lines isolated from patients with certain malignant conditions, as well as in apparently healthy individuals. Several years later, EBV was found to be the causative agent of infectious mononucleosis. Like other herpesviruses, primary infection with EBV can be either "silent" or symptomatic (i.e., infectious mononucleosis). Following primary infections, the virus usually persists for a lifetime as a latent infection, though it can be reactivated, leading

to symptomatic infection. This has been described in immunosuppressed patients. Miller, G. in Virology, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

Infectious mononucleosis

The typical manifestation of symptomatic EBV primary infection is infectious mononucleosis. This disease is primarily one of late adolescence and young adulthood. Initial symptoms are headache, fatigue and malaise, with sore throat occurring in the majority of cases in the first week. Fever lasts for about two to three weeks, high at the onset (39.5°C or above) and decreasing over the last week to ten days. Symptoms of fatigue and low fever may persist for weeks or months after the onset of acute illness. Patients with mononucleosis develop generalized lymphadenopathy, with tenderness of the lymph nodes. Enlargement of the spleen is observed in approximately half of patients; occasionally the liver is also enlarged and tender. A rash occurs in a small number of patients. Central nervous system involvement is occasionally seen. This can be manifested as aseptic meningitis, encephalitis, psychosis, coma, and other syndromes. Fatal mononucleosis has been observed to occur in patients with deficiencies of the immune system, and occasionally in apparently healthy adults.

EBV infection leading to infectious mononucleosis originates and persists in the oropharynx. Nearly all patients shed the virus for weeks to months following infection. B cells probably become infected in the oropharynx region, then circulate the virus. In otherwise healthy individuals, all but a small percentage of infected B cells are cleared from the circulation within several weeks, but it is possible to recover EBV from a small fraction of circulating lymphocytes for the remainder of the patient's lifetime. Autoantibodies also develop in infectious mononucleosis.

Treatment of infectious mononucleosis is usually symptomatic; antivirals such as Acyclovir have minimal effects

and are not generally used. Miller, G. in Virology, p. 1945-1946, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

Chronic active EBV

Chronic active infections are another consequence of EBV, and can be classified into two types. In the severe type, manifestations include pneumonitis, hepatitis, uveitis, and hematologic abnormalities. The disease is usually prolonged, relapsing, and occasionally fatal, either as a result of respiratory failure or of T-cell lymphoma. Patients often have extremely high titers of antibodies to EBV virus particle antigens. The second form of chronic EBV infection, often called "chronic fatigue syndrome," is less well defined in its association with the virus, though some patients also have high EBV antibody titers. Symptoms include fatigue, chronic pharyngitis, tender lymph nodes, headaches, myalgia, and arthralgia. Miller, G. in Virology, p. 1943, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

Sjogren's syndrome and dry eye syndrome

Sjogren's syndrome and dry eye syndrome are two related conditions believed to be associated with EBV infection. Sjogren's syndrome is characterized by dry mouth and dry eyes, associated with lymphoid cell infiltration of the salivary and lacrimal (tear) glands. Kidney, skin, and musculoskeletal symptoms also occur. Non-Hodgkins lymphoma develops in some Sjogren's patients. While Sjogren's is thought to be an autoimmune syndrome, increased levels of EBV DNA have been detected in salivary gland biopsies and blood cells of Sjogren's patients. This suggests that EBV, normally latent in a small number of salivary gland epithelial cells, may be reactivated in Sjogren's patients and provide a target for autoimmune attack. Saito et al., J. Exp. Med., 169:2191-2198 (1989). Dry eye syndrome, which is limited to ocular symptoms, may share this causative mechanism.

Hematologic syndromes

Numerous hematologic syndromes are associated with primary EBV infection. These include thrombocytopenia, neutropenia, hemolytic anemia and aplastic anemia; all are deficiencies of one or more types of mature blood cell.

Burkitt's lymphoma

Burkitt's lymphoma is one of several malignant conditions associated with EBV infection. In certain regions of Africa, Burkitt's lymphoma is the most common tumor of children, with an incidence as high as 1 case per 10,000 people per year. In the majority of young African children with this disease, the primary symptom is a unilateral swelling of the jaw. In older children, the initial clinical symptom is usually an abdominal mass. Occasionally, enlargement of the lymph nodes, liver, and/or spleen is the initial clinical symptom. Leukemic symptoms or central nervous system symptoms may also be seen. The tumors are fast-growing and disease progresses rapidly. When the disease was originally described by Burkitt, the mortality was very high, with 80% of patients dying within a year of diagnosis. While the first-year mortality has now been reduced to 20%, largely through chemotherapy, this disease is still a significant cause of mortality.

The association of Burkitt's lymphoma with EBV is based on several lines of evidence. Over 90% of Burkitt's tumor biopsies from endemic areas of Africa contain EBV DNA, though the viral DNA is not normally found in Hodgkin's disease or non-Hodgkin's lymphoma, for example. Children in Africa who developed Burkitt's lymphoma were shown to have been infected with EBV months to years prior to tumor development, and their antibody titers to EBV antigen were about twice as high as matched controls who did not develop cancer. Once the tumor develops, children have viral antibody titers tenfold higher than healthy children.

Because Burkitt's lymphoma is usually multifocal by the time it is diagnosed, systemic chemotherapy is the usual

treatment, with surgery and radiation therapy often used to reduce tumor size. Burkitt's lymphoma outside the endemic African area does not respond satisfactorily to treatment. Burkitt's tumors of African origin do respond fairly well to cytotoxic drugs, such as cyclophosphamide, methotrexate, and vincristine. However, a major complication occurring early in therapy is "acute tumor lysis syndrome," in which tumor breakdown releases large amounts of cell by-products into the blood, causing acidosis, hyperuricemia, hyperphosphaturia, hyperproteinemia, and hyperkalemia. Sudden death due to cardiac arrhythmia can occur, as can acute kidney failure. Miller, G. in Virology, p. 1946, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

Nasopharyngeal carcinoma

Nasopharyngeal carcinoma (NPC) is a malignancy of the epithelial cells of the nasopharynx. This tumor has one of the highest incidences known for any cancer, due largely to its extraordinarily high frequency in the southern regions of China, and among persons originally from this area. Among women in the Guangdong province, the incidence is 11 cases per 100,000; among males in this region the incidence is 27/100,000. In men from Guangdong aged 40-60, the population in which the disease is most common, the incidence is higher still. The incidence among Chinese emigrating to Taiwan, Hong Kong, Singapore, Malaysia and the United States is somewhat less, but still far higher than in Europe and the United States as a whole, where the incidence is low, approximately 1 in 100,000. NPC begins in the nasopharynx, but often presents as a large cervical mass resulting from spread of the tumor to lymph nodes in the region. Other symptoms include nasal obstruction, postnasal discharge, or symptoms relating to the ear, including hearing loss, tinnitus, or otitis media. Local spread of tumor may cause cranial nerve involvement, headache, or jaw muscle involvement. NPC may metastasize to the liver, lung, skin, lymph nodes and bone.

NPC is usually treated by surgery in combination with high doses of radiation. Survival depends on the extent

of disease progression at time of diagnosis. For tumors confined to the nasopharynx, survival after five years is about 80%. Once the disease begins to spread via the lymph nodes, metastasis is rapid and fewer than 20% of patients survive for five years. Miller, G. in Virology, p. 1946, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

Virtually all undifferentiated NPC biopsy specimens from around the world contain EBV genomes. A single clone of EBV is associated with each tumor. Furthermore, the EBV genome is transcribed into mRNA in the tumors. Well before tumors become clinically detectable, patients have elevated antibody titers to EBV antigens.

Lymphoma

Lymphoproliferative disease is associated with EBV in immunodeficient individuals, though not all B-cell lymphoma in such patients is EBV-associated. Symptoms depend largely on the location of the tumor. Usual sites are mediastinum, pleura, brain, spinal cord, and the abdominal and mesenteric lymph nodes. Nodules in the lung can cause symptoms of pneumonitis. EBV-associated lymphoma occurs in patients with congenital immunodeficiency, such as severe combined immunodeficiency or ataxia telangiectasia, and also with immunodeficiency acquired through immunosuppressive therapy accompanying organ transplantation, or as a result of AIDS. Immunosuppression is thought to increase replication of the virus; EBV excretion increases in the throat of immunosuppressed persons previously infected with the virus, and the linear (replicative) form of the viral genome is found in nearly half of such patients.

In addition to the most common EBV-associated malignancies, (Burkitt's lymphoma, NPC and lymphoma), several other EBV-associated tumors have been described. EBV-associated lymphomas of the brain have occasionally been seen in otherwise healthy individuals, that is, having no known immunodeficiency. EBV has also been found to be associated with epithelial cell tumors of the respiratory tract, and with

Hodgkin's disease.

Several EBV-associated lesions occur in AIDS patients in addition to lymphoma. Lymphocytic interstitial pneumonitis occurs mainly in children; it is chronic and variable in course. Symptoms include hypergammaglobulinemia, high antibody titers to EBV replicative antigens, and poor antibody response to certain EBV antigens. Hairy oral leukoplakia of the tongue is a chronic productive EBV infection of the epithelial cells of the tongue.

Epstein-Barr virus biology and genetics

EBV is a herpesvirus, and like other herpesviruses has a large (172 kb), linear, double-stranded DNA genome. The virus core, toroid in shape, is wrapped with the DNA and surrounded by a protein tegument and an outer envelope with external glycoprotein spikes. The viral genome is arranged with a series of tandemly arranged short terminal direct repeats, a series of tandemly arranged internal direct repeats, and a long and a short unique sequence domain.

EBV is able to immortalize lymphoid cells in culture. The ability of EBV to cause this indefinite lymphocyte proliferation is directly responsible for its role in clinical lymphoproliferative disorders. Viral DNA in immortalized cells exists as circular molecules, or episomes, rather than in virus particles. The virus is then said to be latent.

Immortalized cells express at least nine latent EBV gene products, many or all of which are thought to be involved in the transformation process, though the role of each is not entirely understood. Six of these genes encode nuclear proteins, EBNA-1, -2, -3A, -3B, -3C, and -LP. Two genes encode small, nonpolyadenylated RNAs, EBER-1 and EBER-2. One encodes an integral membrane protein, LMP.

Latently infected cells can be reactivated to active (lytic) infection, in which virus particles are produced. After induction of lytic infection, EBV genes in addition to the latent genes are expressed. Early after induction, a group of viral genes called the immediate early genes, which

include EBNA-LP, EBNA-2 and ZLF1 (which encodes the Z protein), are expressed. At least 30 early lytic genes, including ALF5, which encodes the viral DNA polymerase, are expressed slightly later, after which the 30 or more late viral genes are expressed. The late genes mainly encode structural viral proteins, such as the gp150 capsid protein, or proteins which facilitate viral egress from the infected cells.

Several drugs have been shown to inhibit some aspects of EBV replication *in vitro*, but none has been found to have great clinical efficacy, and no means for control of latent infection has been found. Human α-interferon is capable of inhibiting the stimulation of cellular DNA synthesis and cell proliferation that follows EBV infection of lymphocytes *in vitro*. In clinical trials, the number of renal allograft patients shedding virus following interferon treatment was 38%, compared to 65% in the untreated control patients. Miller, G. in Virology, p. 1945, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

Acycloguanosine (Acyclovir) acts on the lytic phase of EBV replication, inhibiting synthesis of the linear viral genome which would ultimately be packaged into virus particles. However, Acyclovir has no effect on the circular EBV genome found in latent infection, nor does it inhibit immortalization of B-cells by EBV. Acyclovir has minimal effects on the symptoms of mononucleosis; its efficacy has not been proven in clinical trials on lymphoma in immunocompromised patients.

Classical therapeutics has generally focused upon interactions with proteins in efforts to moderate their disease-causing or disease-potentiating functions. Such therapeutic approaches have failed for EBV-associated diseases. The present invention is directed to an alternative approach to the treatment of such diseases, the antisense inhibition of EBV gene expression through the mediation of oligonucleotides. Antisense methodology is the complementary hybridization of relatively short oligonucleotides to

single-stranded mRNA or single-stranded DNA, or even double stranded DNA, such that the normal, essential functions of these intracellular nucleic acids are disrupted. Hybridization is the sequence specific hydrogen bonding of oligonucleotides to Watson-Crick base pairs of RNA or single stranded DNA. Such base pairs are said to be complementary to one another.

The events which disrupt nucleic acid function are discussed by Cohen in *Oligonucleotides: Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1989) who proposes two possible types of terminating events. The first, hybridization arrest, denotes a terminating event in which the oligonucleotide inhibitor binds to the target nucleic acid and thus prevents, by simple steric hindrance, the binding of essential proteins, most often ribosomes, to the nucleic acid. Methyl phosphonate oligonucleotides; P. S. Miller & P.O.P. Ts'O, *Anti-Cancer Drug Design*, Vol. 2, pp. 117-128 (1987); and α-anomer oligonucleotides, Cohen J.S. ed., *Oligonucleotides: Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton FL (1989) are two of the most extensively studied antisense agents which are thought to disrupt nucleic acid function by hybridization arrest.

A second type of terminating event for antisense oligonucleotides involves enzymatic cleavage of the targeted RNA by intracellular RNase H. The oligonucleotide, which must be of the deoxyribo type, hybridizes with the targeted RNA and this duplex activates the RNase H enzyme to cleave the RNA strand, thus destroying the normal function of the RNA. Phosphorothioate oligonucleotides are a prominent example of an antisense agent which operates by this type of terminating event.

Considerable research is being directed to the application of oligonucleotides as antisense agents for therapeutic purposes. Applications of oligonucleotides as diagnostics, research reagents, and potential therapeutic agents require that the oligonucleotides be synthesized in large quantities, be transported across cell membranes or

taken up by cells, appropriately hybridize to targeted RNA or DNA, and subsequently terminate or disrupt nucleic acid function. These critical functions depend on the initial stability of oligonucleotides towards nuclease degradation.

Oligonucleotides modified to exhibit resistance to nucleases, to activate the RNase H terminating event, and to hybridize with appropriate strength and fidelity to targeted RNA (or DNA) are greatly desired for antisense oligonucleotide diagnostics, therapeutics and research with EBV.

OBJECTS OF THE INVENTION

It is an object of this invention to provide oligonucleotides which are capable of hybridizing with RNA of EBV, or the DNA from which it is derived, to inhibit the synthesis or function of said RNA.

It is a further object to provide oligonucleotides which can modulate the expression of EBV through antisense interaction with viral RNA.

Yet another object of this invention is to provide methods of diagnostics and therapeutics for latent or active EBV infection in animals.

A further object of this invention is to provide methods of diagnostics and therapeutics for EBV-associated malignancy in animals.

Methods, materials and kits for detecting the presence or absence of EBV or the EBV genome in a sample suspected of containing it are further objects of the invention.

Novel oligonucleotides are other objects of the invention.

These and other objects will become apparent to persons of ordinary skill in the art from a review of the instant specification and appended claims.

SUMMARY OF THE INVENTION

In accordance with the present invention, methods of modulating the effects of EBV infection are provided. Oligonucleotides having a sequence of nucleotide bases specifically hybridizable with a selected sequence of an EBV

RNA are provided. It is believed that targeting the EBV EBER-1 RNA, EBER-2 RNA, and/or mRNA encoding the EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP, Z, p150 and DNA polymerase proteins will lead to effective antisense therapy with these oligonucleotides. Methods for treating disease states by administering oligonucleotides, either alone or in combination with a pharmaceutically acceptable carrier, to animals suspected of having EBV-associated diseases are provided.

This relationship is commonly denoted as "antisense." The oligonucleotides are able to inhibit the function of RNA -- either its translation into protein, its translocation into the cytoplasm, or any other activity necessary to its overall biological function. The failure of the RNA to perform all or part of its function results in failure of a portion of the genome controlling the normal life cycle of the virus.

It has now been found that oligonucleotides can be designed especially for EBV infections which are effective in diminishing the infection. It is preferred that oligonucleotides have between about 5 and about 50 nucleic acid base units. It is preferred that the oligonucleotide be specifically hybridizable with EBV EBER-1 RNA, EBER-2 RNA, and/or mRNA encoding the EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP, Z, p150 or DNA polymerase protein. The oligonucleotide may be modified to reduce nuclease resistance and to increase their efficacy.

In accordance with preferred embodiments, the RNA is interfered with to an extent sufficient to inhibit establishment or maintenance of latent EBV infection, or EBV replication. Thus, oligonucleotides which are capable of interacting with portions of EBV RNA or DNA are comprehended. Animals suspected of having EBV-associated disease are contacted with an oligonucleotide made in accordance with this invention. In particular, the present invention is believed to be effective in the treatment of latent and active EBV infections and EBV-associated malignancy, either prophylactically or therapeutically.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Antisense oligonucleotides hold great promise as therapeutic agents for the treatment of many human diseases. Oligonucleotides specifically bind to the complementary sequence of either pre-mRNA or mature mRNA, as defined by Watson-Crick base pairing, inhibiting the flow of genetic information from DNA to protein. Numerous recent studies have documented the utility of antisense oligonucleotides as biochemical tools for studying target proteins. Rothenberg et al., *J. Natl. Cancer Inst.*, 81:1539-1544 (1989); Zon, G. *Pharmaceutical Res.*, 5:539-549 (1987). Because of recent advances in oligonucleotide chemistry, synthesis of nuclease-resistant oligonucleotides, and availability of types of oligonucleotides which exhibit enhanced cell uptake, it is now possible to consider the use of antisense oligonucleotides as a novel form of therapeutics.

For therapeutics, an animal suspected of having an EBV infection is treated by administering oligonucleotides in accordance with this invention. Oligonucleotides may be formulated in a pharmaceutical composition, which may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the oligonucleotide. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like in addition to oligonucleotide.

The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including opthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection.

Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the

like may be necessary or desirable. Coated condoms may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

It is to be expected that differences in the DNA of EBV from different species and from different types within a species exist. Thus, it is believed, for example, that the regions of the various EBV species serve essentially the same function for the respective species and that interference with expression of the genetic information will afford similar results in the various species. This is believed to be so even though differences in the nucleotide sequences among the species doubtless exist.

Accordingly, nucleotide sequences set forth in the present specification will be understood to be representational for the particular species being described. Homologous or analogous sequences for different species of EBV are specifically contemplated as being within the scope of this invention.

The present invention employs oligonucleotides for use in antisense inhibition of the function of EBV RNA. In the context of this invention, the term "oligonucleotide" refers to a polynucleotide formed from naturally occurring bases and furanosyl groups joined by native phosphodiester

bonds. This term effectively refers to naturally occurring species or synthetic species formed from naturally occurring subunits or their close homologs. The term "oligonucleotide" may also refer to moieties which function similarly to naturally occurring oligonucleotides but which have non-naturally occurring portions. Thus, oligonucleotides may have altered sugar moieties or inter-sugar linkages. Exemplary among these are the phosphorothioate and other sulfur-containing species which are known for use in the art. In accordance with some preferred embodiments, at least some of the phosphodiester bonds of the oligonucleotide have been substituted with a structure which functions to enhance the ability of the compositions to penetrate into the region of cells where the RNA or DNA whose activity to be modulated is located. It is preferred that such substitutions comprise phosphorothioate bonds, methyl phosphonate bonds, or short chain alkyl or cycloalkyl structures. In accordance with other preferred embodiments, the phosphodiester bonds are substituted with other structures which are, at once, substantially non-ionic and non-chiral, or with structures which are chiral and enantiomerically specific. Persons of ordinary skill in the art will be able to select other linkages for use in practice of the invention.

Oligonucleotides may also include species which include at least some modified base forms. Thus, purines and pyrimidines other than those normally found in nature may be so employed. Similarly, modifications on the furanosyl portion of the nucleotide subunits may also be effected, as long as the essential tenets of this invention are adhered to. Examples of such modifications are 2'-O-alkyl- and 2'-halogen-substituted nucleotides. Some specific examples of modifications at the 2' position of sugar moieties which are useful in the present invention are OH, SH, SCH_3, F, OCH_3, OCN, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$ where n is from 1 to about 10, and other substituents having similar properties.

Such oligonucleotides are best described as being functionally interchangeable with natural oligonucleotides (or

synthesized oligonucleotides along natural lines), but which have one or more differences from natural structure. All such oligonucleotides are comprehended by this invention so long as they function effectively to hybridize with EBV RNA. The oligonucleotides in accordance with this invention preferably comprise from about 5 to about 50 nucleic acid base units. It is more preferred that such oligonucleotides comprise from about 8 to 25 nucleic acid base units, and still more preferred to have from about 12 to 25 nucleic acid base units. As will be appreciated, a nucleic acid base unit is a base-sugar combination suitably bound to adjacent nucleic acid base unit through phosphodiester or other bonds.

The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed, however the actual synthesis of the oligonucleotides are well within the talents of the routineer. It is also well known to use similar techniques to prepare other oligonucleotides such as the phosphorothioates and alkylated derivatives.

In accordance with this invention, persons of ordinary skill in the art will understand that messenger RNA includes not only the information to encode a protein using the three letter genetic code, but also associated ribonucleotides which form a region known to such persons as the 5'-untranslated region, the 3'-untranslated region, the 5' cap region and intron/exon junction ribonucleotides. Thus, oligonucleotides may be formulated in accordance with this invention which are targeted wholly or in part to these associated ribonucleotides as well as to the informational ribonucleotides. In preferred embodiments, the oligonucleotide is specifically hybridizable with a transcription initiation site, a translation initiation site, a 5' cap region, an intron/exon junction, coding sequences or sequences in the 5'- or 3'-untranslated region.

Several genes have been identified as preferred targets for antisense. EBNA-2 has been demonstrated to be essential to the process of immortalization. Viruses from which the EBNA-2 gene is deleted have been found to lack immortalizing properties. Moreover, the lack of a functional EBNA-2 gene results in the lack of expression of other EBV genes including EBNA-1, -3 and EBER-2. Thus EBNA-2 probably initiates a cascade of gene expression. Miller, G. in Virology, p. 1924, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

LMP has also been found to be important in immortalization of cells. When removed from the virus and transformed into rodent cells, it has been shown to result in oncogenic transformation. The LMP gene product can also produce secondary changes in genes that control cell adhesion and morphology. Thus LMP is a likely candidate to play an important role in malignant transformation in EBV-induced malignancy. Miller, G. in Virology, p. 1925, 1929, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

The two EBER genes are the most abundant EBV RNAs in latent infection. Although their function is not known, it can be presumed by their heavy rate of transcription that they play some important role in maintenance of latency. Kieff, E. and D. Liebowitz, in Virology, p. 1895, 2nd ed., B.N. Fields and D.M. Knipe et al., eds. Raven Press, Ltd., NY (1990).

The biochemical function of EBNA-3A, -3B and -3C are also unknown. These three proteins are expressed from genes which have a common origin, and are tandemly located on the EBV genome. The proteins have significant similarities and sequence with some divergence. The EBNA-3 proteins are highly hydrophilic and have been localized to the nucleus where they may influence essential nuclear functions.

EBNA-LP (EBNA-4) is a set of proteins encoded within the leader of the EBNA mRNA and translated from those mRNAs when the first and second exons are spliced to created the

EBNA-LP initiation codon. The protein is localized as coarse granules in the nucleus; *in situ* hybridization shows a linear arrangement and these sites may be involved in EBV RNA metabolism. The proteins electrophorese as a ladder of proteins from 20 to 130 kD, with different size products often produced by the same virus in different cells. No function for LP has been described. Miller, G. in Virology, p. 1929, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990). Kieff, E. and D. Liebowitz, in Virology, p. 1898, 2nd ed., B.N. Fields and D.M. Knipe et al., eds. Raven Press, Ltd., NY (1990).

Thus, although the EBV genome contains at least 84 potential open reading frames, we have determined the 8 genes selected above to be essential for maintenance of latent EBV in cells and, therefore, good targets for therapeutic intervention.

The ZLF1 gene product, called Z, is a key immediate early transactivator of early EBV lytic gene expression. The Z protein, sometimes acting in combination with another protein (R), has been shown to transactivate most EBV early lytic promotors studied to date. Transactivation apparently affects transcription of early genes by interaction with enhancer elements in the promotor regions. Z is a DNA binding protein which binds directly to AP1-like sequences. Its expression enhances its own synthesis, and directly transactivates every EBV strong early lytic promotor studied to date. Kieff, E. and D. Liebowitz, in Virology, p. 1903, 2nd ed., B.N. Fields and D.M. Knipe et al., eds. Raven Press, Ltd., NY (1990). This key role of ZLF1 in lytic EBV infection makes it a good candidate for antisense inhibition of EBV.

A number of EBV genes are required for viral DNA replication. These genes are believed likely to be good targets for antisense inhibition of the virus. EBV DNA polymerase, while not required for establishment of episomal transformation, is required for replication of viral DNA during lytic infections. Inhibition of expression of the ALF5 gene encoding EBV DNA polymerase should result in a reduction

of EBV genomic copies and virus replication. For this reason, it too is a good candidate for inhibition of EBV infection.

The major capsid protein in EBV, p150, is encoded by the cLF1 gene. This is known as a result of homology comparisons with the HSV capsid gene and because *in vitro* translation of cLF1 mRNA yields a protein identical in size with the 150-kD major EBV capsid protein. Thus, inhibition of expression of cLF1 may result in failure of the virus to package and release infectious particles. Kieff, E. and D. Liebowitz, in Virology, p. 1906, 2nd ed., B.N. Fields and D.M. Knipe et al., eds. Raven Press, Ltd., NY (1990). For this reason, cLF1 is yet another good gene target for antisense inhibition.

Oligonucleotides useful in the invention are complementary to EBV RNA, DNA (especially for oligonucleotides directed to intron/exon junctions) or to the corresponding messenger RNA (mRNA) or premessenger RNA. Thus, the oligonucleotides in accordance with the invention preferably have one of the sequences shown in Table 1 and Table 2, or an effective portion thereof. Thus, it is preferred to employ any of these oligonucleotides as set forth above or any of the similar oligonucleotides, which persons of ordinary skill in the art can prepare from knowledge of the preferred antisense targets for the modulation of the viral infection.

TABLE 1

ANTISENSE OLIGONUCLEOTIDES TARGETED TO EBV

Gene	Target site	Oligo sequence (antisense)	SEQ ID NO
EBNA-2	AUG-1	ATA GAA TGT AGG CAT GAT GGC	1
	AUG-2	GTA GGC ATG ATG GCG GCA GAT	2
EBNA-3A	AUG	CCT TGT CCA TTT TGT CTG CA	3
	Splice junction	ACG GCA ACA AAT TAC TTA CC	4
EBNA-3B	Splice junction	TCT AGG TGC CTC TTA CCA AC	5
	Splice junction	CTT ACC AAC ATG GAA TCA TA	6
EBNA-3C	Splice junction	CTT GCA GGT GCG ATT GCT AA	7
	Splice junction	CTA AAA TTA ATG GAA ACA TG	8
EBNA-LP	AUG-1	CGG TCT CCC ATT TGT GTG GAC	9
	AUG-2	TCA CTT CGG TCT CCC ATT TGT	10
LP/EBNA	E/I Splice junction	ACT TAC CCC TGA AGG TGA AC	11

	I/E Splice junction	CTC CCC TAG GCT TGG ATG GC	12
	E/I Splice junction	ACT TAC CTC TGG CCC GAT ACC	13
	I/E Splice junction	CGA GGG GAC CCT GAG ACG GGT	14
EBER-1	Internal sequence	CCT AGC AAA ACC TCT AGG GC	15
	Internal sequence	GAC GGC AGA AAG CAG AGT CT	16
EBER-2	Internal sequence	GGT GTG TCC GAA ACC ACT AG	17
	Internal sequence	AGG CGG GAA GCC TCT CTT CT	18
ALF5 (DNA Polymerase)	AUG	TCC CCC AGA CAT GGT TAA AA	19
ZLF-1	AUG	GTC CAT CAT CTT CAG CAA AG	20
cLF-1 (gp150)	AUG	TTG AGG CCA TGA CAC AAG GT	21

TABLE 2
ANTISENSE OLIGONUCLEOTIDES TARGETED TO EBV

Gene	Target site	Oligo sequence (antisense)	SEQ ID NO
BBLF4 (Helicase/ATPase)	AUG	CTC CTC GGC CAT TCC TGT TCA	22
BBLF3 (Helicase/Primase)	AUG	GCC GGC TGG CAT CCT CCG AGT	23
BBLF2 (ATPase/ori binding)	AUG	CCT TTA AAA CAT CCG GTC ACG	24
BGLF4 (protein kinase)	AUG	ATT CAC ATC CAT GTT CCT CAA	25
BALF2 (ss DNA binding protein)	AUG	TGC ACC CTG CAT GAT GCC CAA	26
BALF5 (DNA polymerase)	AUG	TCC CCC AGA CAT GGT TAA AAC	27
BORF2 (Ribonucleotide reductase)	AUG	GGT CGT TGC CAT GCT GTG TGG	28
BARF1 (Ribonucleotide reductase)	AUG	CAA CTT GGA CAT GGC AAT AAA	29
BMRF1 (ds DNA binding protein)	AUG	AGT GGT TTC CAT GAT CAC AAG	30
BSLF1 (Helicase/primase)	AUG	GGG GGC GGA CAT TAT TTA AGA	31

BBRF1 (Virion glycoprotein) AUG CAT GTT GAA CAT GAC CTC AGA 32

BBRF2 (Virion glycoprotein) AUG CAC CCT AGG CAT CGT GGA GAT 33

The oligonucleotides of this invention can be used in diagnostics, therapeutics and as research reagents and kits. For therapeutic use, the oligonucleotide is administered to an animal suffering from a EBV infection. It is generally preferred to apply the therapeutic agent in accordance with this invention internally, such as intravenously, transdermally or intramuscularly. Other forms of administration, such as topically or intralesionally, may also be useful. Inclusion in suppositories is presently believed to be likely to be useful. Use of the oligonucleotides of this invention in prophylaxis is also likely to be useful. Such may be accomplished, for example, by providing the medicament as a coating in condoms and the like. Use of pharmacologically acceptable carriers is also preferred for some embodiments.

The present invention is also useful in diagnostics and in research. Since the oligonucleotides of this invention hybridize to nucleic acid from EBV, sandwich and other assays can easily be constructed to exploit this fact. Provision of means for detecting hybridization of oligonucleotide with EBV or EBV nucleic acid present in a sample suspected of containing it can routinely be accomplished. Such provision may include enzyme conjugation, radiolabelling or any other suitable detection systems. Kits for detecting the presence or absence of EBV may also be prepared.

The following specific examples are given for illustrative purposes only and are not intended to limit the invention.

EXAMPLES

Example 1

Immortalization of lymphocytes: Cells are grown in RPMI 1640 medium with 10% fetal bovine serum added for recently transformed lymphoblastoid cell lines, and 5% calf serum added for the B95-8 cell line. Primary human B lymphocytes are isolated from peripheral blood by centrifugation over Renografin-Ficoll, adherence of monocytes to plates for 1 hour in complete medium, removal of nonadherent cells to plates

coated with rabbit anti-human immunoglobulin, and removal and discarding of non-adherent primary T cells after 2 hours. EBV viral stocks are obtained from supernatants of B95-8 infected marmoset cell lines following induction of virus replication with 20 ng of 12-O-tetradecanoylphorbol-13-acetate (TPA) and 3 mM sodium butyrate for 5 days. Primary B lymphocytes, 5 x 10^7 cells per plate, are incubated for 2 hours with 8 ml of virus stock. Stock is replaced with fresh medium and the cells are incubated overnight, removed from the plate and cultured for 5 days. Knutson, J.C., *J. Virol.* 64:2530-2536 (1990). Efficiency of transformation is determined by measuring the number of transformed cells by endpoint dilution in a microtiter plate. The fraction of transformed cells is the ratio of microwells that show transformation to the total number of cells exposed to virus at the lowest dilution that shows transformants. Results are corrected for the plating efficiency of cell populations that consist entirely of immortalized cells. Miller, G. in Virology, p. 1923, 2nd Ed., B. N. Fields and D.M. Knipe, eds., Raven Press, Ltd., NY (1990).

Example 2

Inhibition of lymphocyte immortalization by antisense oligonucleotides: To assess the ability of oligonucleotides to inhibit lymphocyte immortalization by EBV, cells are resuspended in 2 ml of medium containing 10 μM concentrations of oligonucleotide. After incubation for 18 hours, cells are rinsed in medium and infected with EBV as described in Example 1. Transformation efficiency is determined as in Example 1.

Example 3

Induction of lytic phase: Lytic EBV infection is studied by activating latently infected B lymphoblasts to a state of permissiveness for virus replication using inducers of replication. Kieff, E. and D. Liebowitz, in Virology, p. 1902, 2nd ed., B.N. Fields and D.M. Knipe et al., eds. Raven Press, Ltd., NY (1990). 12-O-Tetradecanoylphorbol-13-acetate (TPA) is one of the most reproducible and broadly active inducers of virus replication. To achieve induction, TPA is

added to cells 48 hours before harvest, to a final concentration of 20 ng/ml.

Example 4

MTT assay for lytic viral infection: Following induction of viral replication as in Example 3, viral yields are titered using the immortalization assay of Example 1 as a quantitative assay. Alternatively, extent of cell lysis is quantitated using the MTT assay. This method measures cell viability and is based on the reduction of the tetrazolium salt, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to MTT formazan by mitochondrial enzymes of viable host cells (Mossmann, T., J. Immunol. Methods. 65:55 (1983)). Cells are treated with MTT followed by SDS to dissolve the crystals of MTT formazan. The blue color of the MTT formazan is measured spectrophotometrically at 570 nm on an automated plate reader. Viability is determined by comparing the absorbance (optical density, O.D.) of each drug cytotoxicity control with the mean O.D. of the cell control cultures and expressed as percent of control.

Example 5

Oligonucleotide inhibition of induction of lytic EBV infection: Cells are grown as in Example 1, pretreated with oligonucleotide as in Example 2, and infected with virus as in Example 1. Following immortalization by virus, viral replication is induced using phorbol esters as in Example 3. During the five-day culture period after induction, cells are posttreated with oligonucleotide by replacing medium with fresh medium containing 10 μM oligonucleotide on day 1 and day 3. Lysis of host cells is quantitated using the MTT method as described in Example 4. Reduction in cell lysis (i.e., reduction in viral replication) is determined by comparison to controls without oligonucleotide treatment.

Example 6

Assay for proliferation of NPC-derived cell lines containing EBV: Epithelial tumor cell lines are established from nasopharyngeal carcinoma biopsy specimens as described by Glaser et al., *Proc. Natl. Acad. Sci. U.S.A.*, 86:9524-9528

(1989). Pieces of biopsy tissue are immediately placed into RPMI 1640 medium. Tissue is cut or teased into fragments approximately 1 mm^3 in size and seeded into 25-ml tissue culture flasks. Cells are incubated at 37°C and passaged twice weekly. Epithelial cells along with a few fibroblasts are expected to grow after 3 days. Epithelial cells usually grow steadily and exhibit a loss of contact inhibition when cultured in this manner. Clonal isolates are assayed for EBV infection by indirect immunofluorescence using EBNA-positive human autoimmune serum, and by Southern blot analysis to look for the EBV genome. EBV-positive clones are cultured and used in this assay; positive clones remain stably infected. Transformation is assayed according to the method of Tomei et al., *Nature*, 329:73-75 (1987), which defines transformation as the ability of human epithelial cells to grow in soft (0.3%) agar. Plates are seeded with 100,000 viable cells and colonies are counted at day 15. Primary human epithelial cells normally give 0-50 colonies per 100,000 cells in soft agar medium; upon transformation, this number can increase to over 1000 colonies per 100,000 cells.

Example 7

Antisense inhibition of proliferation of NPC-derived cell lines: To assess the ability of oligonucleotides to inhibit proliferation of NPC-derived cell lines, clonal cultures of EBV-positive NPC-derived cells are fed with medium containing 10 μM concentrations of oligonucleotide. After incubation with oligonucleotide for 18 hours, treated cells and untreated controls are seeded on soft agar as in Example 6. Transformation efficiency is determined as in Example 6, and percent inhibition of proliferation of oligonucleotide-treated cells relative to untreated controls is calculated.

Example 8

Induction of tumorigenesis in mice with nasopharyngeal carcinoma cells: Tumorigenicity of NPC-derived cells is measured by injection of tumor cell suspension subcutaneously into athymic nude mice essentially as described by Blair, Science, 10:1122-1125 (1982). NPC biopsy tissue is

homogenized, resuspended at 2.5 X 10^7 viable cells per ml, and 0.2 ml of cell suspension is injected subcutaneously into 4-5 week old Swiss female athymic nude mice. Lombardi et al., Cell, 49:161-170 (1987). Animals are monitored for tumor formation for 6-8 weeks after injection; tumor formation is expected at 2-4 weeks postinjection, and is confirmed by standard histological methods.

Example 9

Oligonucleotide inhibition of nasopharyngeal carcinoma cell-induced tumorigenesis in mice: Tumors are induced in athymic nude mice by injection of NPC tumor-derived cells as in Example 6. The oligonucleotides in Table 1 and Table 2 are tested for ability to inhibit tumorigenesis as follows: 50-μl doses of oligonucleotide in PBS (either 0.25 or 2.5 mg oligonucleotide) are injected into the site of NPC injection twice a day for two to four weeks, or until control mice (without oligonucleotide injection) have developed tumors. Tumor size in treated and control mice is compared at one, two, three and four weeks after tumor induction, and the average percentage reduction in tumor size as a result of oligonucleotide treatment is calculated (cure= 100% reduction).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Kevin Anderson, David Ecker

(ii) TITLE OF INVENTION: Compositions And Methods For Treatment Of Epstein-Barr Virus-Associated Diseases

(iii) NUMBER OF SEQUENCES: 33

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(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 Mb STORAGE

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: PC-DOS

(D) SOFTWARE: WORDPERFECT 5.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: n/a

(B) FILING DATE: herewith

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

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(C) REFERENCE/DOCKET NUMBER: ISIS-0474

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (215) 568-3100

(B) TELEFAX: (215) 568-3439

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATAGAATGTA GGCATGATGG C 21

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTAGGCATGA TGGCGGCAGA T 21

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCTTGTCCAT TTTGTCTGCA 20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ACGGCAACAA ATTACTTACC 20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TCTAGGTGCC TCTTACCAAC 20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTTACCAACA TGGAATCATA 20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTTGCAGGTG CGATTGCTAA 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CTAAAATTAA TGGAAACATG 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGGTCTCCCA TTTGTGTGGA C 21

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TCACTTCGGT CTCCCATTTG T 21

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ACTTACCCCT GAAGGTGAAC 20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CTCCCCTAGG CTTGGATGGC 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ACTTACCTCT GGCCCGATAC C 21

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CGAGGGGACC CTGAGACGGG T 21

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CCTAGCAAAA CCTCTAGGGC 20

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GACGGCAGAA AGCAGAGTCT 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGTGTGTCCG AAACCACTAG 20

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AGGCGGGAAG CCTCTCTTCT 20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TCCCCCAGAC ATGGTTAAAA 20

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GTCCATCATC TTCAGCAAAG 20

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TTGAGGCCAT GACACAAGGT 20

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CTCCTCGGCC ATTCCTGTTC A 21

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GCCGGCTGGC ATCCTCCGAG T 21

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CCTTTAAAAC ATCCGGTCAC G 21

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ATTCACATCC ATGTTCCTCA A 21

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TGCACCCTGC ATGATGCCCA A 21

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TCCCCCAGAC ATGGTTAAAA C 21

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GGTCGTTGCC ATGCTGTGTG G 21

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CAACTTGGAC ATGGCAATAA A 21

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

AGTGGTTTCC ATGATCACAA G 21

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GGGGGCGGAC ATTATTTAAG A 21

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CATGTTGAAC ATGACCTCAG A 21

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CACCCTAGGC ATCGTGGAGA T 21

WHAT IS CLAIMED IS:

1. An oligonucleotide comprising from 5 to 50 nucleic acid base units specifically hybridizable with a selected nucleic acid sequence encoding EBER-1, EBER-2, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP, Z, p150 or DNA polymerase.

2. The oligonucleotide of claim 1 specifically hybridizable with at least a portion of the AUG region and comprising CAT.

3. The oligonucleotide of claim 1 specifically hybridizable with at least a portion of the 3' untranslated region, the 5' untranslated region, the 5' cap, or an intron/exon splice junction.

4. The oligonucleotide of claim 1 in a pharmaceutically acceptable carrier.

5. The oligonucleotide of claim 1 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.

6. The oligonucleotide of claim 1 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a phosphorothioate moiety.

7. The oligonucleotide of claim 1 wherein at least one of the nucleotide bases is modified.

8. The oligonucleotide of claim 7 wherein the base is modified at the 2' position.

9. The oligonucleotide of claim 8 wherein the 2' modification is OH, SH, SCH_3, F, OCH_3, OCN, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$ where n is from 1 to about 10.

10. An oligonucleotide comprising at least a portion of one of the sequences identified in Table 1 and Table 2.

11. The oligonucleotide of claim 10 in a pharmaceutically acceptable carrier.

12. The oligonucleotide of claim 10 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.

13. The oligonucleotide of claim 10 wherein at

least one of the linking groups between nucleotide units of the oligonucleotide comprises a phosphorothioate moiety.

14. The oligonucleotide of claim 10 wherein at least one of the nucleotide bases is modified.

15. The oligonucleotide of claim 14 wherein the base is modified at the 2' position.

16. The oligonucleotide of claim 15 wherein the 2' modification is OH, SH, SCH_3, F, OCH_3, OCN, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$ where n is from 1 to about 10.

17. A method for modulating the activity of Epstein-Barr virus comprising contacting the virus with an oligonucleotide having 5 to 50 nucleic acid base units specifically hybridizable with a selected nucleic acid sequence encoding EBER-1, EBER-2 RNAs, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP, Z, p150, or DNA polymerase.

18. The method of claim 17 wherein the oligonucleotide is specifically hybridizable with at least a portion of the AUG region and comprises CAT.

19. The method of claim 17 wherein the oligonucleotide is specifically hybridizable with at least a portion of the 3' untranslated region, the 5' untranslated region, the 5' cap, or an intron/exon splice junction.

20. The method of claim 17 wherein the oligonucleotide is in a pharmaceutically acceptable carrier.

21. The method of claim 17 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.

22. The method of claim 17 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a phosphorothioate moiety.

23. The method of claim 17 wherein at least one of the nucleotide bases of the oligonucleotide is modified.

24. The method of claim 23 wherein the base modification is at the 2' position.

25. The method of claim 24 wherein the 2' modification is OH, SH, SCH_3, F, OCH_3, OCN, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$ where n is from 1 to about 10.

26. A method for modulating the activity of Epstein-Barr virus comprising contacting the virus with an oligonucleotide comprising at least a portion of one of the sequences identified in Table 1 and Table 2.

27. A method for treating an EBV-associated disease comprising contacting an animal suspected of having an EBV-associated disease with a therapeutically effective amount of an oligonucleotide having from 5 to 50 nucleic acid base units specifically hybridizable with a selected nucleic acid sequence encoding EBER-1, EBER-2, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP, Z, p150, or DNA polymerase.

28. The method of claim 27 wherein the oligonucleotide is specifically hybridizable with at least a portion of the AUG region and comprises CAT.

29. The method of claim 27 wherein the oligonucleotide is specifically hybridizable with at least a portion of the 3' untranslated region, the 5' untranslated region, the 5' cap, or an intron/exon splice junction.

30. The method of claim 27 wherein the oligonucleotide is in a pharmaceutically acceptable carrier.

31. The method of claim 27 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.

32. The method of claim 27 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a phosphorothioate moiety.

33. The method of claim 27 wherein at least one of the nucleotide bases of the oligonucleotide is modified.

34. The method of claim 33 wherein the base modification is at the 2' position.

35. The method of claim 34 wherein the 2' modification is OH, SH, SCH_3, F, OCH_3, OCN, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$ where n is from 1 to about 10.

36. The method of claim 27 wherein the EBV-associated disease is selected from the group consisting of nasopharyngeal carcinoma, a latent EBV infection, an active EBV infection, a malignancy, Burkitt's lymphoma, Sjogren's

syndrome, dry eye syndrome, infectious mononucleosis, chronic active EBV infection, lymphoma, lymphocytic interstitial pneumonitis, hairy oral leukoplakia of the tongue, and a hematologic syndrome.

37. A method for treating an EBV-associated disease comprising contacting an animal suspected of having an EBV-associated disease with a therapeutically effective amount of an oligonucleotide comprising at least a portion of one of the sequences identified in Table 1 and Table 2.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/08989

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/70; C07H 21/00, 21/04
US CL :514/44; 536/27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE
SEARCH TERMS: ANTISENSE OLIGONUCLEOTIDES, EPSTEIN-BARR VIRUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Fields et al, eds. , "VIROLOGY", Second Edition, published 1990 by Raven Press, Ltd., (New York), pages 1889 - 1920, see esp. pages 1889 - 1892.	1 - 37
Y	Journal of the National Cancer Institute, Volume 81, No. 20, issued 18 October 1991, Rothenberg et al., "Oligodeoxynucleotides as Anti-sense Inhibitors of Gene Expression: Therapeutic Implications," pages 1539 - 1544, see esp. pages 1541 - 1544.	1 - 37
Y	Journal of Virology, Volume 64, No. 6, issued June 1990, Joyce C. Knutson, "The Level of c-fgr RNA Is Increased by EBNA-2, an Epstein-Barr Virus Gene Required for B-Cell Immortalization," pages 2530 - 2536, see esp. page 2530, column 1, first paragraph.	1 - 37

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
08 DECEMBER 1992	28 DEC 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer GARY L. KUNZ

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,806,463 (Goodchild et al.) 21 February 1989, see columns 1 - 2 and claims 1 - 12.	1 - 37